ELSEVIER

Fish & Shellfish Immunology 23 (2007) 71−85

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

# RAG-1 and IgM genes, markers for early development of the immune system in the gadoid haddock, *Melanogrammus aeglefinus*, L.

Y. Corripio-Miyar [a,b,*], S. Bird [a], J.W. Treasurer [b], C.J. Secombes [a]

[a] *Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen AB24 2TZ, Scotland, UK*
[b] *Viking Ardtoe Marine Laboratory, Ardtoe, Acharacle, Argyll PH36 4LD, Scotland, UK*

Received 22 June 2006; revised 22 September 2006; accepted 26 September 2006
Available online 4 October 2006

**Abstract**

The full sequence of the heavy chain of Immunoglobulin M (IgM) and a partial fragment of the recombination activating gene-1 (RAG-1) gene were sequenced in haddock, *Melanogrammus aeglefinus*, L. The complete transcript of haddock IgM consisted of 1865 bp and translated into a 572-aa peptide. The RAG-1 fragment was 1776 bp and was identified as the core region of RAG-1. These two immune genes were used in expression studies as markers of early development in haddock larvae. A DIG labelled oligoprobe of the RAG-1 gene was used in whole-mount *in situ* hybridisation (WISH). A hybridisation signal for RAG-1 was first detected in larvae at 25 days post-hatching (dph) in two bilateral symmetric regions of the head identified as the thymus. Further expression studies were carried out by RT-PCR analysis of RAG-1 and IgM on larval samples obtained during early development, i.e. from fertilisation to weaning. Haddock RAG-1 expression was detected after 21 days post-fertilisation (dpf) whilst IgM transcripts were not detected until 40 dpf, equivalent to day 29 post-hatching. These results suggest that the immune system in haddock starts to develop in larvae of 6−7 mm in length (25−29 dph).
© 2006 Elsevier Ltd. All rights reserved.

*Keywords:* Haddock; RAG-1; IgM; Whole-mount *in situ* hybridisation; Gene expression

## 1. Introduction

Gadoids, cod and haddock, in particular, are species with a high economic importance in the North Atlantic communities [1,2]. Over the last few decades, due to the severe decline of the natural stocks [3,4], great interest has been generated in gadoid aquaculture. During the early 1990's, initial studies on cod farming proved the feasibility of the culture of this species in countries like Canada, Norway, Iceland and Scotland [5,6]. More recently, haddock has been the focus of considerable interest in the fish farming industries of Canada and Scotland. However, the production of

* Corresponding author. Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen AB24 2TZ, Scotland, UK. Tel.: +44 1224 272870; fax: +44 1224 272396.
*E-mail address:* y.corripio@abdn.ac.uk (Y. Corripio-Miyar).

1050-4648/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2006.09.006

haddock in aquaculture is still in its infancy and research to date has predominantly focused on larval rearing, nutrition and photoperiod studies [7–10].

Currently, there is a paucity of information regarding haddock health management that needs to be addressed so as to increase the survival of the cultured fish and avoid the use of antibacterial drugs [11]. Thus, it is important to establish the earliest time that fish can be vaccinated, as the production of juveniles is one of the main difficulties present in the aquaculture industry, primarily due to the high mortalities observed throughout early development [12]. However, a problem exists for fish that are immunised at a very early age before they are immunocompetent, as there is a risk of inducing immunological tolerance, resulting in an actual decrease in the secondary response [13–15].

The ontogeny of the immune organs in teleosts has been widely studied in different species, such as yellowtail, Japanese flounder, seabream and turbot [16–18]. Most of these morphological studies have been carried out using histology as the main technique to investigate the development of the immune organs in fish. However, immune genes are also good markers in the study of the physiological maturity of the immune system and potentially give a more accurate measure of the initial development of immunocompetence than for example the appearance of lymphocytes in a lymphoid organ [19]. Genes like *Ikaros*, recombination activating genes-1 (RAG-1) and RAG-2 involved in different stages of the development of lymphocytes have been used to follow the development of lymphoid organs in fish [20,21]. The RAG-1 gene is essential in the differentiation of immature B and T cells and is expressed in primary lymphoid organs, although not in mature cells [22]. It is this expression during the maturation of the lymphoid organs that makes the RAG-1 gene such a useful marker for the study of the development of these organs [23,20,24]. Immunoglobulin M (IgM) is also an important gene that can be used in the study of the ontogenesis of the immune system, as it is the first formed antibody of the primary response in higher vertebrates [25]. The presence of IgM has been extensively reported in fish species [26–28], including studies of its appearance during development and even maternal transfer of IgM to the offspring. The latter is not seen in all teleosts and, in fish like cod, immunohistochemical studies with polyclonal and monoclonal antibodies to IgM, showed no transfer of maternal IgM to the offspring of this particular species [29]. In the absence of maternal Ig, presumably such fry have to produce endogenous Ig as quickly as possible. Knowledge acquired from expression studies of RAG-1, IgM and other genes involved in teleost development can provide vital information regarding the ontogeny of the immune system in different fish species, with implications for vaccination regimes. Here, we describe the cloning and sequencing of the IgM gene and a partial RAG-1 gene in haddock and study the expression in larvae of RAG-1 using whole-mount *in situ* hybridisation and both genes using RT-PCR.

## 2. Materials and methods

### 2.1. Haddock

Haddock, *Melanogrammus aeglefinus*, were obtained from Viking Ardtoe Marine Laboratory, Scotland (Former Sea Fish Industry Authority, Marine Farming Unit). Fish of 500–750 g were used for harvesting tissues for RNA extraction and were maintained at the aquarium facilities of the Zoology building, University of Aberdeen, in a recirculation unit supplied with saltwater at 9–10 °C in semi-darkness. The fish were fed commercial pellets once every two days.

Egg and larval materials were collected from hatchery reared broodstock during the spawning season of 2002 in order to study the expression of the IgM and RAG-1 genes during early development by RT-PCR. A pool of 500 eggs per day was sampled at two-day intervals after fertilisation and during egg incubation, 0 day post-fertilisation (dpf) to 12 dpf. Around hatching, a pool of 100 larvae each was sampled on a daily interval for a period of 5 days (12–16 dpf) after which pools of 50 larvae were sampled approximately at 5-day intervals until larvae were weaned to dry food (45 dpf). All samples were stored in 1–2 ml of RNAzol™ (Biogenesis, Friendswood, Texas) at −80 °C until RNA extraction was performed.

A single batch of haddock eggs stage V [30], from hatchery reared broodstock during the spawning season of 2003, was used for whole-mount *in situ* hybridisation. Larvae were disinfected (15 s dip in 2 ppt Kick Start®, Cid Lines, Belgium) and set up in a single tank containing 4000 L seawater (UV sterilised, 1 μm filtered) in a Controlled Environment Room (air and water temperature, photoperiod and light intensity controlled throughout) at the Viking Ardtoe Marine Laboratory hatchery. The temperature of the room was maintained between 8.6 and 9.7 °C for the duration of

Download English Version:

https://daneshyari.com/en/article/2433628

Download Persian Version:

https://daneshyari.com/article/2433628

Daneshyari.com